REPORT TITLE

Toxicology Response by the Endosulfan Task Force to the Health Effects Division Risk Assessment for the Endosulfan Reregistration Eligibility Decision Document Dated February 17, 2000

Requirement for a Developmental Neurotoxicity Study and Assessment of Additional FQPA Safety Factor for Endosulfan

DATA REQUIREMENT

Not Applicable

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SUBMISSION VOLUME

Volume 2 of 3

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d)(1)(A), (B), or (C).

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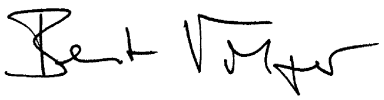
Date: January 4, 2001

STATEMENT OF GOOD LABORATORY PRACTICE

The following response is not subject to the principles of 40 CFR 160, Good Laboratory Practice Standards, as promulgated in Federal Register 54, No. 158, 34067-34704, August 17, 1989.



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Date: January 4, 2001

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HEALTH EFFECTS DIVISION (HED) RISK ASSESSMENT FOR THE ENDOSULFAN REREGISTRATION ELIGIBILITY DECISION DOCUMENT, DATED FEBRUARY 17, 2000

TOXICOLOGY CHAPTER

RE: Endosulfan: HED Risk Assessment for the Endosulfan RED Document (DP Barcode: D250471; Memo by Stephen C. DeVito, Ph.D., dated February 17, 2000) - Exposure Assessment, Section 3.0 "Hazard Characterization" and Related Documents;

Endosulfan 079401: Toxicology Chapter for the Reregistration Eligibility Document (HED memo by Nicole C. Paquette, Ph.D. dated November 22, 1999).

The Endosulfan Task Force (ETF), comprised of Aventis CropScience, FMC, and Makhteshim-Agan North America, respectfully submit the following three volumes in response to the above referenced draft chapter. There are three key areas of concern regarding the EPA's review of the endosulfan toxicity data that the ETF will address. These areas are:

- The NOAEL selection for the 21-day dermal study in rats (Volume 1)
- Requirement of a developmental neurotoxicity study and retention of a FQPA safety factor of 3x due to uncertainty associated with this data gap (Volume 2)
- EPA's suggestion that endosulfan may be an endocrine disruptor (Volume 3)

This volume specifically addresses the requirement for a developmental neurotoxicity study and the subsequent retention of an additional FQPA safety factor.

I. INTRODUCTION

In preparation for the final Reregistration Eligibility Decision (RED) on the active ingredient endosulfan, the EPA Health Effects Division (HED) provided the Endosulfan Task Force (ETF) with a draft of their human health risk assessment for all registered uses of this chemical. Supporting documents for this risk assessment included the Hazard Identification Assessment Review Committee (HIARC) Toxicology Chapter, the HIARC report on toxicological endpoints for risk assessment, and the FQPA Safety Factor Committee report. On May 10, 2000, the ETF submitted an initial 30-day response identifying errors in the draft risk assessment and providing brief summaries on issues of concern regarding the selection of toxicological endpoints, application of FQPA safety factors and implications regarding the potential of endosulfan to be an endocrine disruptor.

The purpose of this submission is to further elucidate the areas of concern discussed briefly in the 30-day response. One of the key issues previously identified by the ETF was HED's decision to require a developmental neurotoxicity (DNT) study, and to assess endosulfan an additional FQPA safety factor in the absence of this data. HED made this decision based upon the recommendation

of the FQPA Safety Factor Committee, which was in opposition to two HIARC determinations that held the DNT in reserve pending results from a subchronic neurotoxicity study.

> *"…. The HIARC determined that there is: 1) no indication of increased susceptibility of rats or rabbit fetuses to in utero exposure in the developmental toxicity study for endosulfan; 2) quantitatively, no indication of increased susceptibility to rat offspring following pre- and/or post-natal exposure in reproductive study; and 3) no evidence of adverse effects on the developing fetal nervous system in any of these studies. Therefore, the HIARC, using a tiered approach, placed the requirement for a developmental neurotoxicity study in reserve pending the receipt of the subchronic neurotoxicity study.*
>
> *However, the FQPA Safety Factor Committee concluded that it was appropriate to request the developmental neurotoxicity study in rats at this time because the subchronic neurotoxicity study will only address the neuropathological concerns in adults and not the concern for effects in developing fetuses. The developmental neurotoxicity study is requested at this time because of the concern for: 1) the fetal effects reported in the open literature abstract (Lakshmana et al., 1994); and 2) the severity of effects seen in the female offspring of the F_0 generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats.*

The ETF does not concur with the FQPA Safety Factor Committee (SFC) recommendation or the subsequent HED decision concerning the need for a DNT. The ETF strongly believes that the available data is complete, reliable and adequate to determine that a DNT is not required for endosulfan. In the following sections the ETF has provided a summary and evaluation of the available data demonstrating a lack of support for the conclusions made by the FQPA SFC. In addition, the ETF would recommend that EPA follow their current guidance regarding the requirement for a DNT, and assess the available endosulfan data against the triggers EPA agreed would be used for this determination.[1] The remainder of this document will provide a review of EPA's criteria for the requirement of a DNT and a weight-of-evidence evaluation of the neurotoxicity of endosulfan as it relates to these triggers.

II. EVALUATION OF THE ENDOSULFAN TOXICITY PROFILE

As stated previously, HED recommended, based on the findings of the FQPA SFC, that a DNT be required for endosulfan. The rationale provided by the FQPA SFC for their recommendation to require the DNT focused on two key points: 1) concern for effects on developing fetuses based on results from a study in the public literature (Lakshmana and Raju, 1994); and 2) pituitary and uterine weight changes seen in the reproductive toxicity study. The ETF believes that a science-based weight-of-evidence evaluation of the available data does not support the FQPA SFC rationale for recommending a DNT study for endosulfan, or the assessment of an additional safety factor.

[1] Markis S., *A Retrospective Analysis of Twelve Developmental Neurotoxicity Studies Submitted to the USEPA Office of Prevention, Pesticides, and Toxic Substances (OPPTS).* Draft 11/12/98. Appendix A-1.

A. Developing Fetuses – Potential for Adverse Effects

The endosulfan database has been reviewed twice (Sept 98 and Jan 00) by the Hazard Identification Assessment Review Committee (HIARC) and once by the FQPA Safety Factors Committee (Feb 99). All three reviews were consistent in the determination that the available guideline data do not show any indication of increased sensitivity to children or infants from pre- and postnatal exposures to endosulfan.

Results from the HIARC reviews were as follows:

"*Determination of Susceptibility*

The database is complete and there are no data gaps pertaining to developmental or reproductive toxicity. The data provided no indication of increased sensitivity of rats or rabbits to in utero and post-natal exposure to endosulfan. Two prenatal developmental toxicity studies, one in rats and one in rabbits failed to show evidence of developmental toxicity in the absence of maternal toxicity. In the two-generation reproduction study in rats, effects in the offspring were observed only at or above treatment levels that resulted in evidence of parental toxicity."[2]

"FQPA Considerations

Based on hazard assessment, the HIARC recommended to the FQPA Safety Committee, that 10X factor for the protection of infants and children should be reduced to 3X because:

> *1) developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following in utero exposures in rats and rabbits;*
>
> *2) the two generation reproduction toxicity study in rats showed no increased susceptibility in pups when compared to adults; and*
>
> *3) there was no evidence of abnormalities in the development of fetal nervous system in the pre/post natal studies. Neither brain weight nor histopathology (perfused or non-perfused) of the nervous system was affected in the subchronic and chronic toxicity studies."*[3]

The FQPA SFC concluded the following:

*"The FQPA Safety Factor Committee concluded that the **FQPA safety factor** is required, however can be **reduced to 3x** because: 1) there is no evidence of increased susceptibility in any study…"*[4]

[2] D.S. Liem and J. Rowland (memo), ENDOSULFAN – Report of the Hazard Identification Assessment Review Committee. PC Code: 079401. Dated October 7, 1998. (p. 19)
[3] N.C. Paquette memo Endosulfan 079401: Toxicology Chapter for the Reregistration Eligibility Document. Dated November 22, 1999. p. 26
[4] B. Tarplee Memo Endosulfan – Report of the FQPA Safety Factor Committee. PC Code 079401. Dated 20-Nov-1998. P.5

Yet the FQPA SFC stated in their rationale for requiring a DNT, "*concern for effects in developing fetuses."* This comment was most likely based on results from a single public literature paper (Lakshmana and Raju, 1994). The paper claims potential loss of cognitive function in 25-day old rats that had been gavaged with endosulfan from post-natal days 2-25. The toxicological significance of this finding is unclear. The study was performed at a dose level of 6 mg/kg/day that has been shown in the rat developmental, reproductive and neurotoxicity studies to be a toxic dose, especially in females. So while the author indicated a lack of significant weight change between the control and treated groups, there is no data provided to show whether the slowed response to food stimulus was due to actual effects on cognitive function, or a secondary result of general systemic toxicity manifested as a decrease in appetite, activity or other clinical signs. There was also no information regarding difference in response between males and females, where the significance in effect may have been due primarily to toxicity in the females. Before this data is considered in the weight-of-evidence evaluation for endosulfan, there should be a scientific review to determine the significance of the purported results. In the HIARC review (dated October 7, 1998), the committee specifically recommended that **"this study be reviewed/evaluated and that a DER be prepared**." Therefore, the ETF believes that a valid weight-of-evidence determination, using key guideline studies, demonstrates sufficient evidence that endosulfan is not a developmental neurotoxicant and does not support the FQPA SFC statement regarding a "*concern for effects in developing fetuses*."

B. Evaluation of Organ Weight Effects in the Reproductive Toxicity Study

The FQPA SFC also cited in their rationale for requiring the DNT that effects seen in pups in the reproductive toxicity study were of concern:

"*… the severity of effects seen in the female offspring of the F_0 generation (increased pituitary) and F_1b generation (increased uterine weights) at the high-dose when compared to the toxicity observed in parental animals (decreased body weight) at this dose in the two-generation reproduction study in rats."*

This conclusion was contrary to the HIARC reviews of the reproductive toxicity study that stated:

"The offspring effects were not considered to be severe when compared to the maternal effects, since it was seen only in one generation (not consistent) and these were not the target organ for toxicity in other studies with endosulfan."[5]

"The effects at the high dose level cannot be considered as an indicator of any special sensitivity to the pups, because the biological relevance of these effects is unclear"[6]

This conclusion was also in conflict with the FQPA SFC's own recommendation for reducing the FQPA safety factor to 3x because "*1) there is no evidence of increased*

[5]Ibid., p. 18.
[6] N.C. Paquette, p. 18.

susceptibility in any study; 2) the severity of the fetal effects in the reproduction study were not consistent between generations and the target organ toxicity seen in this study was not seen in any other study."

The ETF believes that a thorough evaluation of the data clearly demonstrates that the effects seen on the pituitary and uterus in the reproductive study are not of toxicological significance. As stated by both the HIARC and the FQPA SFC, the effects were not consistent across generations, did not show a dose-related trend, neither the pituitary or the uterus were target organs of toxicity in any other endosulfan studies, and there was no supporting histopathological changes. In addition, there is further evidence to suggest that these effects were not of significance for determination of potential effects on developing fetuses:

1) Pituitary weight change in F_0 generation pups

An evaluation of the individual animal data shows that the statistically significant difference in the pituitary weights of the F_0 first mating group was due to a single animal (#245) whose organ weight (0.0109 g) was 2.5x greater in weight than the average pituitary weight in the rest of the high dose group animals (0.0044 g).

2) Uterine Weight Changes

There have been four uterotrophic assays performed with endosulfan, all were negative for weight change.

Table 1. Endosulfan: *In vivo* Uterotrophic Assays

Type of *in vivo* study	**Endpoints**	**Endocrine Effects**
Uterotrophic assay in sexually immature Sprague-Dawley rats (3 mg/kg/day i.p. on day 18-20 of age) (Wade et al. 1997)	Uterus: growth, peroxidase activity, number of PR/ER; Pituitary: weight, hormones (GH, prolactin, TSH, LH, FSH); Serum: Thyroxin	No uterotrophic activity or hormonal changes. DES caused increase in uterus weight (80%), peroxidase, prolactin and a decrease in number of ER
Uterotrophic assay in sexually immature CD 1-mouse (10 mg/ kg bw/day s.c. on days 17 -19 of age) (Shelby et al. 1996)	Uterine growth	No increase in uterine wet mass. DES**,** E_2, (4-OH)-tamoxifen, DDT, methoxychlor were positive
Uterotrophic assay in sexually immature AP-Wistar rats (5 - 100 mg/kg bw/day s.c. for 3 days) (Ashby et al. 1997)	Uterine growth	No increase in uterine wet mass. Estradiol and methoxychlor were clearly positive.
Uterotrophic assay on young ovariectomized female Wistar rats (Raizada et al. 1991)	Uterus / cervix / vagina wet weight and glycogen content; pituitary weight; histology	No effects after gavage of 1.5 mg/kg bw/day for 30 days although transient clinical signs were present.

Based on this weight-of-evidence, neither the pituitary or uterine weight effects are of toxicological significance and do not suggest a potential for endosulfan to effect the developing

fetus. Therefore, the ETF concludes that the available endosulfan data adequately addresses the FQPA SFC's points of concern and does not support the requirement for a DNT.

III. CRITERIA FOR THE REQUIREMENT OF A DEVELOPMENTAL NEUROTOXICITY STUDY

In 1998 the EPA's Health Effects Division (HED) established the primary triggers for requirement of a developmental neurotoxicity study.[1] "*The requirement of the developmental neurotoxicity testing for pesticides is based on whether the chemical profile meets one or more of the following criteria.*

The substance has been shown to:

- *Cause CNS malformation following prenatal exposure;*
- *Affect brain weight in offspring, which does not appear to be related solely to general growth retardation, following pre- and/or postnatal exposure;*
- *Cause neuropathology in developing or adult animals or neuropathy in humans;*
- *Cause persistent functional changes in the offspring which may be the result of effects on the nervous system;*
- *Act to significantly modify hormonal responses associated with the development of the nervous system, leading to significant developmental effects (e.g., effects on sexual maturation)."*

In addition, a weight-of-evidence assessment of the database is conducted, and all information pertinent to the assessment of neurotoxicity potential of the chemical is considered when determining the need for a developmental neurotoxicity study. This could include factors such as:

a) Acute behavioral/functional changes are produced in adult animals by an effect of the compound on the nervous system;
b) The compound exhibits a structure-activity relationship to a known neurotoxicant or neuroactive chemical;
c) Evidence of developmental toxicity to fetal tissues, organs, and/or systems (other than the CNS) generates concern regarding potential effects on functional development of affected fetuses; or
d) The potency of the chemical, the persistence of neurotoxic effects, or the partitioning of effects in the animal model (e.g. brain cholinesterase inhibition that occurs at a much lower dose than elicits plasma cholinesterase inhibition) generates an additional level of concern.

The ETF believes that the available data for endosulfan adequately addresses the above mentioned areas of concern, and does not meet the criteria necessary to trigger a DNT study.

A. Evaluation of Relevant DNT Criteria

The ETF assessed the developmental and reproductive toxicity data as they relate to EPA's DNT criteria listed in Section II. Criteria of concern would be:

- *Cause CNS malformation following prenatal exposure;*
- *Affect brain weight in offspring, which does not appear to be related solely to general growth retardation, following pre- and/or postnatal exposure;*
- *Cause neuropathology in developing or adult animals or neuropathy in humans;*
- *Cause persistent functional changes in the offspring which may be the result of effects on the nervous system;*
- *Act to significantly modify hormonal responses associated with the development of the nervous system, leading to significant developmental effects (e.g., effects on sexual maturation).*
- *Evidence of developmental toxicity to fetal tissues, organs, and/or systems (other than the CNS) generates concern regarding potential effects on functional development of affected fetuses.*

There was no indication from the reproductive or developmental toxicity study that prenatal exposure to endosulfan had any effect on central nervous system morphology nor was there any evidence of peripheral neuropathies. There were no effects on brain weight in the offspring of either generation (see Table 8, Appendix 1). There was no indication of functional deficits in the offspring of either generation in the reproductive toxicity study, and the acute neurotoxicity study showed no effects on the functional observation battery or motor activity as a result of endosulfan exposure. The effects on the pituitary and uterus were not consistent across generations, showed no dose-related trend and were not associated with effects in any other reproductive organs. Therefore, there is no evidence to support the supposition by HIARC that these effects may be due to hormonal perturbation. Lastly, there were no developmental effects in either the rat or rabbit, which would generate concern regarding the potential effects on the functional development of affected fetuses, and no evidence from the reproductive study to demonstrate the potential for long-term functional effects.

In summary, based on the information provided by the developmental and reproductive toxicity studies, there is no indication that endosulfan produces central nervous system malformation, brain weight effects, persistent functional changes, effects on sexual maturation or any other developmental toxicity as a result of pre- or post-natal exposures. Nor was there any evidence that endosulfan exposure resulted in any neuropathological effects in developing animals. Therefore, endosulfan does not trigger any of the above-mentioned criteria for requirement of a DNT.

B. Evaluation of Neurotoxicity Data

The ETF also assessed the neurotoxicity data as they relate to EPA's DNT criteria listed in Section II. Criteria of concern would be:

a) *Acute behavioral/functional changes are produced in adult animals by an effect of the compound on the nervous system;*
b) *The compound exhibits a structure-activity relationship to a known neurotoxicant or neuroactive chemical; or*
c) *The potency of the chemical, the persistence of neurotoxic effects, or the partitioning of effects in the animal model (e.g. brain cholinesterase inhibition that occurs at a much lower dose than elicits plasma cholinesterase inhibition) generates an additional level of concern.*

As noted in the HIARC review of the acute neurotoxicity study for endosulfan, there was "*No compound-related effects on motor activity were noted for rats that survived. No treatment-related effects were seen on: the rearing frequency, fore-and hind-limb grip strength, and on landing foot-spread; body weight and food consumption; organ weight; gross pathology; or histo(neuro) pathology*."[7] Therefore, there is no indication that endosulfan affects behavior or functional capabilities following acute exposures.

Since the mechanism of endosulfan is thought to be associated with inhibition of gamma-amino-butyric acid (GABA) receptors, it will exhibit structure-activity relationships with other GABA inhibiting neurotoxic insecticides. However, as has been shown repeatedly in this review, there is no indication that this mechanism of action results in effects on the developing nervous system. As concluded by HIARC, *"there was no evidence of abnormalities in the development of fetal nervous system in the pre/post natal studies. Neither brain weight nor histopathology (perfused or non-perfused) of the nervous system was affected in the subchronic and chronic studies."*[8]

Lastly, there is no indication from the data that endosulfan causes any persistence of neurotoxic effects or partitioning of effects in the animal which would generate an additional level of concern.

Therefore, based on an evaluation of the relevant neurotoxicity data as they relate to the criteria for requirement of a DNT study, endosulfan does not trigger this requirement.

[7] Ibid., p. 19
[8] Ibid., p. 26

CONCLUSIONS

Following a thorough evaluation of the available data on endosulfan, the ETF concludes that the available data adequately addresses the concern for sensitivity to children and infants, and does not meet any of the HED triggers for a developmental neurotoxicity study. This conclusion is based on the following key points:

- Both the FQPA Safety Committee and HIARC concluded that "*based on the results of animal studies conducted under OPPTS guidelines there is no evidence of increased sensitivity or susceptibility of the fetus, infants or children to the toxicity of endosulfan."* [9]
- HED's FQPA Safety Factor Committee also concluded that "*…1) there is no evidence of increased susceptibility in any study;* [and] *2) the severity of the fetal effects in the reproduction study were not consistent between generations and the target organ toxicity seen in this study was not seen in any other study; ..."* [10]
- HED's draft RED chapter on toxicology stated that "*The effects* [pituitary and uterine weights] *at the high dose level cannot be considered as an indicator of any special sensitivity to the pup, because the biological relevance of these effects is unclear."*
- Additionally, weight effects of the pituitary gland and uterus in the F_0 and F_1b pups, respectively, could not be correlated to any resulting histopathology; were not considered severe when compared to the maternal effects by the HIARC Committee; and did not affect any developmental or reproductive endpoints in either generation.
- Endosulfan does not trigger any of the criteria established by EPA to require a DNT:
  - A second evaluation by the HIARC further determined that "*there was no evidence of abnormalities in the development of the fetal nervous system in the pre/post natal studies. Neither brain weight nor histopathology (perfused or non-perfused) of the nervous system was affected in the subchronic and chronic toxicity studies."* [11]
  - The acute neurotoxicity study showed no evidence of neuropathology, nor were there any adverse effects on motor activity, rearing frequency, fore- or hind-limb grip strength, or landing foot –spread.
- GLP studies should provide a greater weight-of-evidence in the determination of sensitivity to infants and children, than a non-GLP public literature abstract which has not undergone a thorough review by the Agency.

Based on this conclusion, the ETF request that HED remove the requirement for a DNT study and the resulting additional 3 x safety factor from the endosulfan RED.

[9] Endosulfan: HED Risk Assessment for the Endosulfan Reregistration Eligibility Decision (RED) Document. Chemical No. 079401. Case No. 0014. Barcode D250471. Dated February 17, 2000. p. 3

[10] B. Tarplee Memo Endosulfan – Report of the FQPA Safety Factor Committee. PC Code 079401. Dated 20-Nov-1998. P.5

[11] N.C. Paquette memo Endosulfan 079401: Toxicology Chapter for the Reregistration Eligibility Document. Dated November 22, 1999. p. 26

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APPENDIX 1

Reproductive Toxicity Study Summary

Title: **Effect of Endosulfan Technical (Code: HOE 02671 OI AT209) on Reproductive Function of Multiple Generations in the Rat**

Laboratory : Report HST 204/83768 (A29428); EPA MRID 00148264

Experimental work : From 4/21/1982 to 12/13/1983

Test material : HOE 02671 OI AT209, purity 97%

Methodology : MAFF (Japan, Jan1985), EPA FIFRA (Nov 1984)

GLP conformity : Yes

Material and Methods:

Four groups of 32 male and 32 female Crl: COBS CD® (SD) BR rats received endosulfan technical continuously via the diet at concentrations of 0, 3, 15, and 75 ppm for 10 weeks pre-mating and throughout mating, gestation, and lactation. The F1 animals selected to remain on study as the next generation (28/sex/group) were offered diets at the same concentrations as their parents from weaning for at least 10 weeks before mating, and throughout mating, gestation, and lactation of the F2 litters. Clinical observations, body weights, body weight changes, water and food consumption, reproduction, and litter data were recorded.

According to food consumption throughout the treatment period, group mean achieved dosage were as follows:

Dose (ppm)	**3**	**15**	**75**
Female Dose (mg/kg/day)	0.2	1.2	6.2
Male Dose (mg/kg/day)	0.2	1.0	5.0

Summary of effects:

1. Clinical signs

F0: There were no test material-related clinical observations for F0 adults given 3, 15, or 75 ppm or F1 offspring from any of the treated groups.

F1: There were no test material-related clinical observations for F1 adults given 3, 15, or 75 ppm or F2 offspring from any of the treated groups.

2. Mortality

F0: Single mortalities in females occurred in the control group and at 3 and 15 ppm. There were no mortalities at 75 ppm in either the males or females.
F1: There was single female death in the F1B generation in the control group. There were no mortalities in any of the other dose groups.

3. Bodyweight

At 75 ppm F0 generation females and both F1 males and females showed marginally lower mean weekly weight gains, and during gestation at first mate of both generations in comparison with controls. Among F0 females the difference was statistically significant ($p<0.05$) at week 4 only. There were no other statistically significant differences and F0 males at 75 ppm showed slightly higher weight gain than among control animals.

4. Food consumption

Food consumption in the F1 males at 75 ppm showed slightly lower values throughout the dosing period. No other dose groups were effected.

5. Reproduction Data

F0: There were no effects noted on mating performance, pregnancy rate or gestation periods at any dose.
F1: There were no effects noted on mating performance, pregnancy rate or gestation periods at any dose.

Table 1: Fertility Indices in F0 generation

Dose Level		0 ppm	3 ppm	15 ppm	75 ppm
First Mating					
Number of paired females	N	32	32	32	32
Total number inseminated	N	31	32	29	32
	%	97	100	91	100
Total number pregnant	N	31	29	27	31
	%	100	91	93	97
Fertility index Number pregnant/ N° paired	%	97	91	84	97
Second Mating					
Number of paired females	N	32	32	31	32
Total number inseminated	N	31	31	29	32
	%	97	97	94	100
Total number pregnant	N	31	31	29	32
	%	100	100	100	100
Fertility index Number pregnant/ N° paired	%	97	97	94	100

Table 2: Fertility Indices in F1B generation

Dose Level		0 ppm	3 ppm	15 ppm	75 ppm
First Mating					
Number of paired females	N	28	28	28	28
Total number inseminated	N	27	26	26	27
	%	96	93	93	96
Total number pregnant	N	27	26	25	26
	%	100	100	96	96
Fertility index Number pregnant/ N° paired	%	96	93	89	93
Second Mating					
Number of paired females	N	28	28	28	28
Total number inseminated	N	27	28	27	28
	%	96	100	96	100
Total number pregnant	N	27	28	26	27
	%	100	100	96	96
Fertility index Number pregnant/ N° paired	%	96	100	93	96

6. Litter Data

F0: There were no treatment-related effects on litter loss, litter size, pup mortality, sex ratios or mean pup weights. At 75 ppm during lactation to weaning there was a decrease in mean litter weights during both mates, with occasional statistically significant differences ($p<0.05$). However, there was no corresponding effect on pup weight or litter size.

F1: There were no treatment-related effects on litter loss, litter size, pup mortality, sex ratios or mean litter and pup weights.

7. Organ weights

- Relative, but not absolute, liver weights were increased in both male ($p<0.05$) and female ($p<0.01$) F0 adults at 75 ppm. Relative liver weights were also increased in F1B adult females at 15 ppm ($p<0.01$) and 75 ppm ($p<0.001$).
- Relative, but not absolute, increase in heart weight was seen in F0 males at 15 ppm ($p<0.05$) and 75 ppm ($p<0.01$).
- Relative, but not absolute, increase in kidney weights were in F0 and F1b males at 75 ppm ($p<0.01$)
- Relative, but not absolute, brain weight was increased in F0 females at 75 ppm ($p<0.05$)
- Relative, but not absolute, pituitary weight was increased in F0 females of the 1st mating at 75 ppm ($p<0.05$).
- Relative, but not absolute, uterine weight was increased in the F1B females of the 1st mating at 75 ppm ($p<0.01$).

Table 3: Group Mean Liver Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)	25.66	26.74	26.50	28.35	14.03	14.08	13.70	14.96
Relative weight[1]	26.18	25.97	27.07	28.03*	13.82	13.93	13.81	15.20**
F_0 Weanlings (1st mating)								
Absolute weight (g)	2.57	2.42	2.42	2.50	2.47	2.22	2.34	2.41
Relative weight[1]	2.45	2.42	2.53	2.51	2.34	2.28	2.40	2.42
F_0 Weanlings (2nd mating)								
Absolute weight (g)	2.80	2.63	2.54	2.71	2.66	2.56	2.51	2.54
Relative weight[1]	2.71	2.58	2.60	2.79	2.57	2.51	2.57	2.61
F_1 Adults								
Absolute weight (g)	25.86	27.18	24.91	26.23	13.12	13.68	14.10	14.82
Relative weight[1]	25.86	26.12	25.30	26.90	13.18	13.50	14.22**	14.82***
F_1 Weanlings (1st mating)								
Absolute weight (g)	1.83	2.18	1.78	1.77	1.60	2.00	1.68	1.68
Relative weight[1]	1.96	1.89	1.80	1.90	1.72	1.75	1.69	1.77
F_1 Weanlings (2nd mating)								
Absolute weight (g)	2.13	2.30	2.08	2.19	2.04	2.30	1.96	2.04
Relative weight	2.16	2.16	2.13	2.26	2.08	2.09	2.07	2.13

Table 4: Group Mean Pituitary Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)	0.016	0.017	0.017	0.017	0.020	0.018	0.019	0.019
Relative weight[1]	0.016	0.016	0.018	0.017				
F_0 Weanlings (1st mating)								
Absolute weight (g)	0.004	0.003	0.004	0.003	0.004	0.004	0.003	0.005
Relative weight[1]	0.003	0.003	0.004	0.003	0.004	0.004	0.003	0.005*
F_0 Weanlings (2nd mating)								
Absolute weight (g)	0.003	0.003	0.004	0.003	0.003	0.004	0.003	0.003
Relative weight[1]	0.003	0.003	0.004	0.004				
F_1 Adults								
Absolute weight (g)	0.018	0.017	0.016	0.017	0.018	0.020	0.021	0.017
Relative weight[1]								
F_1 Weanlings (1st mating)								
Absolute weight (g)	0.003	0.003	0.003	0.003	0.003	0.004	0.003	0.003
Relative weight[1]	0.003	0.002	0.003	0.003	0.003	0.003	0.003	0.003
F_1 Weanlings (2nd mating)								
Absolute weight (g)	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003
Relative weight								

[1]values adjusted for body weight as covariate

Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

Table 5: Group Mean Uterus Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)					0.633	0.623	0.578	0.591
Relative weight[1]								
F_0 Weanlings (1st mating)								
Absolute weight (g)					0.047	0.051	0.050	0.047
Relative weight[1]					0.045	0.052	0.051	0.048
F_0 Weanlings (2nd mating)								
Absolute weight (g)					0.054	0.056	0.057	0.047
Relative weight[1]					0.052	0.055	0.058	0.048
F_1 Adults								
Absolute weight (g)					0.616	0.632	0.645	0.589
Relative weight[1]								
F_1 Weanlings (1st mating)								
Absolute weight (g)					0.035	0.045	0.039	0.043
Relative weight[1]					0.037	0.041	0.039	0.044**
F_1 Weanlings (2nd mating)								
Absolute weight (g)					0.049	0.053	0.049	0.046
Relative weight					0.050	0.050	0.051	0.047

Table 6: Group Mean Ovaries Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)					0.087	0.091	0.094	0.089
Relative weight[1]					0.086	0.090	0.094	0.090
F_0 Weanlings (1st mating)								
Absolute weight (g)					0.020	0.020	0.019	0.021
Relative weight[1]					0.019	0.020	0.019	0.021
F_0 Weanlings (2nd mating)								
Absolute weight (g)					0.021	0.021	0.019	0.020
Relative weight[1]					0.021	0.021	0.019	0.020
F_1 Adults								
Absolute weight (g)					0.082	0.087	0.084	0.086
Relative weight[1]					0.083	0.087	0.084	0.086
F_1 Weanlings (1st mating)								
Absolute weight (g)					0.012	0.016	0.014	0.013
Relative weight[1]					0.013	0.014	0.014	0.014
F_1 Weanlings (2nd mating)								
Absolute weight (g)					0.017	0.018	0.016	0.017
Relative weight					0.017	0.017	0.017	0.017

[1]values adjusted for body weight as covariate,
Significantly different from control, *p<0.05, **p<0.01, ***p<0.001.

Table 7: Group Mean Testes Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)	4.94	4.92	4.81	4.83				
Relative weight[1]	4.95	4.90	4.83	4.82				
F_0 Weanlings (1st mating)								
Absolute weight (g)	0.284	0.269	0.253	0.269				
Relative weight[1]	0.270	0.269	0.266	0.272				
F_0 Weanlings (2nd mating)								
Absolute weight (g)	0.325	0.308	0.298	0.308				
Relative weight[1]	0.314	0.302	0.306	0.317				
F_1 Adults								
Absolute weight (g)	4.64	4.63	4.78	4.66				
Relative weight[1]	1.73	1.72	1.75	1.73				
F_1 Weanlings (1st mating)								
Absolute weight (g)	0.205	0.246	0.205	0.205				
Relative weight[1]	0.217	0.219	0.208	0.217				
F_1 Weanlings (2nd mating)								
Absolute weight (g)	0.260	0.261	0.242	0.248				
Relative weight	0.262	0.246	0.247	0.256				

Table 8: Group Mean Brain Weights (g)

	Male				Female			
Dose (ppm)	**0**	**3**	**15**	**75**	**0**	**3**	**15**	**75**
F_0 Adults								
Absolute weight (g)	2.061	2.090	2.078	2.085	1.851	1.859	1.855	1.883
Relative weight[1]	2.064	2.085	2.081	2.083	1.845	1.854	1.858	1.890*
F_0 Weanlings (1st mating)								
Absolute weight (g)	1.366	1.371	1.339	1.358	1.348	1.289	1.330	1.307
Relative weight[1]	1.350	1.370	1.354	1.361	1.326	1.300	1.339	1.310
F_0 Weanlings (2nd mating)								
Absolute weight (g)	1.423	1.409	1.419	1.421	1.389	1.377	1.335	1.356
Relative weight[1]	1.412	1.403	1.427	1.431	1.378	1.371	1.342	1.365
F_1 Adults								
Absolute weight (g)	2.104	2.109	2.057	2.086	1.947	1.914	1.919	1.958
Relative weight[1]	2.104	2.098	2.061	2.093	1.948	1.909	1.922	1.958
F_1 Weanlings (1st mating)								
Absolute weight (g)	1.315	1.341	1.328	1.294	1.259	1.285	1.271	1.258
Relative weight[1]	1.335	1.297	1.331	1.314	1.280	1.241	1.273	1.277
F_1 Weanlings (2nd mating)								
Absolute weight (g)	1.395	1.399	1.388	1.382	1.351	1.369	1.332	1.318
Relative weight	1.398	1.382	1.394	1.390	1.355	1.340	1.349	1.328

[1]values adjusted for body weight as covariate

Significantly different from control, *p<0.05, **p<0.01, ***p<0.001

9. Macroscopic pathology

F_0 Animals: A slight increased incidence both of animals showing enlarged livers and of animals showing enlarged kidneys was seen in males at 75 ppm.

F_1 Animals: No treatment-related effects were noted in any animals.

10. Microscopic pathology

There was no indication of treatment-related histopathological changes in tissue examined from F1B adults and F2B weanlings.

11. Conclusions

The NOAEL for parental toxicity was 15 ppm (1.2 mg/kg/day), and the parental LOAEL was 75 ppm (6.2 mg/kg/day) based on decreased body weight. The reproductive and developmental NOAEL was 75 ppm (6.2 mg/kg/day), the highest dose tested. A statistically significant increase in pituitary weights in the F_0 females from the first mating at 75 ppm was due to a single animal and was not supported by any histopathological changes. A statistically significant increase in uterine weight in the high dose females of the F1b 1^{st} mating, was not supported by histopathological change, was not seen in any other generation, and was not seen as a target organ in any other study. Therefore, these effects were not considered toxicologically significant.

APPENDIX 2

Rat Developmental Toxicity Study Summary

Title:	**Endosulfan – Testing for Embryotoxicity in the Wistar Rat after Oral Administration**
Laboratory:	Pharma Development Central Toxicology, Hoechst Aktiengesellschaft. Hoechst Study Report No. A51695; EPA MRID 43129101
Experimental work:	From 11 February 1993 to 19 April 1993
Test material:	Hoe 002571 00 Zd98 0005, Batch C 0239 1276, purity 97,3%
Methodology:	MAFF (Japan, Jan1985), EPA FIFRA (Nov 1984)
GLP conformity:	Yes

Material and Methods:

Four groups of 20 female Wistar rats of the Hoe: WISKf (SPF71) strain received Hoe 002571 (endosulfan) in sesame oil orally via gavage at concentrations of 0, 0.66, 2.0 and 6.0 mg/kg bw/day daily from gestation days 7 to 16. The dams were killed and delivered by caesarean section on gestation day 21. The fetuses were then examined morphologically for developmental disturbances.

Summary of effects:

1. Clinical signs
Four dams in the 6.0 mg/kg bw/day group died. One died on day six of treatment, one on day eight and two on day ten. Three dams experienced tonoclonic convulsions for two or three days before death and one had a blood-crusted nose on the day on which it died. The fourth dam died without exhibiting any specific clinical signs of toxicity. Of the dams that survived at 6.0 mg/kg bw/day, 13 had tonoclonic convulsions, which emerged after 4 to 7 treatments between days 10 and 13 of gestation, and persisted from one to three days. Three of these dams also exhibited intermittent increased salivation. One dam showed hyperactivity on day 8 of gestation.

No clinical signs of toxicity were noted in the other dose groups.

2. Mortality
Four pregnant dams in the 6.0 mg/kg bw/day group died. One died on day six of treatment, one on day eight and two on day ten. Two non-pregnant dams in the high dose group also died on test days 14 and 17.

3. Bodyweight
There was significant decrease in body weight gain in dams at 6.0 mg/kg/bw/day during the first week of treatment only. Weights in the other two dose groups were not affected by treatment.

4. Food consumption
Food consumption was significantly reduced in the 6.0 mg/kg bw/day group during the first week of treatment and slightly reduced in the second week.

5. Developmental Effects
One dam in the 2.0 mg/kg bw/day and one in the 6.0 mg/kg bw/day group showed signs of early resorptions. There was no treatment-related effect on the number of corpora lutea/dam, implantations/dam, live fetuses/dam, dead fetuses/dam, pre- or post-implantation losses, litter weight, fetal body weight, or fetal crown-rump length in any dose group.

6. Organ weights
There were no significant changes in organ weight in any dose group.

7. Macroscopic pathology
There were no significant dose-related effects in any of the treated dams. One control dam had a clearly demarcated, flesh-colored thickening in the subcutis of the right groin. Two control dams and one dam in the 2.0 mg/kg bw/day group showed moderate or marked dilatation of one or both renal pelves. One dam in the 6.0 mg/kg bw/day showed a rough and uneven surface of the spleen.

8. Morphological Findings in the Fetuses
There was a statistically significant increase in the number of high dose fetuses with fragmented thoracic vertebrae centra compared to control (6.3% vs. 0.7%) and slightly outside the historical range of 0 – 3.9%. However, this occurrence was seen in the presence of significant maternal toxicity.

9. Conclusions
The maternal toxicity NOAEL was 2.0 mg/kg bw/day, based on increased mortality, decreased body weight gains and food consumption, and clinical signs of toxicity seen at 6.0 mg/kg bw/day. The developmental NOAEL was also 2.0 mg/kg bw/day, based on a slight increased incidence in fragmented thoracic vertebral centra at 6.0 mg/kg bw/day.

APPENDIX 3

Rabbit Developmental Toxicity Study Summary

Title:	**Teratology Study with FMC 5462 in Rabbits**
Laboratory:	Raltech Study Report No. 80070; A23192; EPA MRID 00094837
Experimental work:	From 29 January 1981 to 17 July 1981
Test material:	FMC 5462, purity 97,3%
Methodology:	MAFF (Japan, Jan1985), EPA FIFRA (Nov 1984)
GLP conformity:	Yes

Material and Methods:

Four groups of 20 female New Zealand white rabbits received FMC 5462 (endosulfan technical) in corn oil orally via gavage at concentrations of 0, 0.3, 0.7 and 1.8 mg/kg bw/day daily from gestation days 6 to 28. The does were killed and delivered by caesarean section on gestation day 29. The fetuses were then examined morphologically for developmental disturbances.

Summary of effects:

1. Clinical signs
Four does in the 1.8 mg/kg group showed signs of noisy and rapid breathing, hyperactivity and convulsions. One of these animals (0074) died on day 10 of treatment.

No clinical signs of toxicity were noted in the other dose groups.

2. MORTALITY
Four does in the 1.8 mg/kg bw/day group died on gestation day 7, 10, 21 and 29. Three of deaths were due to improper gavage. The fourth doe died on day 29 of treatment, while awaiting necropsy. A probable cause of death was not established, but black tar-like material was present in the intestine, and the liver and kidney were reported to have a pale appearance. Histopathological examination of these tissues revealed vacuolization of the hepatocytes. This finding was considered incidental and is associated with a variety of systemic disturbances. No deaths occurred in other dose groups.

3. BODYWEIGHT
There were no significant changes in body weight gain for any treated group.

4. Food consumption
Food consumption was not affected for any group.

5. DEVELOPMENTAL EFFECTS
Pregnancy maintenance, implantation, litter size, sex ratio, mean fetal weight and length, and number and percent of live and resorbed fetuses were not significantly different from control for any treated group. There were no dead fetuses in any treatment group or in the control group.

6. Organ weights
There were no significant changes in organ weight in any dose group.

7. Macroscopic pathology
No major gross, soft tissue or skeletal malformations occurred in any treatment group. A single incidence of craniofacial malformation was reported in the control group.

No gross external observations were reported in the high or low dose groups. The only observation in the mid-dose group was a kinked tail that occurred in two fetuses in one litter.

8. MORPHOLOGICAL FINDINGS IN THE FETUSES
The only soft tissue abnormality in the high dose group was the observation of the left carotid artery arising from the innominate; this anomaly was also observed in the control group. Observations in the mid-dose group included enlarged auricles and an accessory left subclavian artery. No observations were reported in the low dose group.

Common skeletal variations and minor anomalies were present in all treatment groups and the control group in a non-treatment-related pattern.

9. Conclusions
The maternal toxicity NOAEL was 0.7 mg/kg bw/day, based on increased mortality and clinical signs of toxicity seen at 1.8 mg/kg bw/day. The developmental NOAEL was also 1.8 mg/kg bw/day, the highest dose tested.